

34506104.ST25.txt SEQUENCE LISTING

<110> Niles, Andrew Maffitt, Mark Haak-Frendscho, Mary

 $<\!120\!>$ RECOMBINANT PROTEOLYTIC TRYPTASES, ACTIVE SITE MUTANTS THEREOF, AND METHODS OF MAKING SAME

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<150> 09/079,970 <151> 1998-04-15
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Asp Val Lys Asp Leu Ala Ala Leu Arg Val Gln Leu Arg Glu Gln His

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Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr 115 120 125

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Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly 180 185 190

Asp Ser Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln 195 200 205

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Cys	Gly	Gly 35	Ser	Leu	Ile	His	Pro 40	Gln	Trp	Val	Leu	Thr 45	Ala	Ala	His
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Thr Val Thr Leu Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro 115 120 125

Cys Trp Val Thr Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro 130 135 140

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Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu 85 90 95

Leu Glu Glu Pro Val Lys Val Ser Ser His Val His Thr Val Thr Leu 100 105 110

Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr 115 120 125

Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Phe Pro 130 135 140

Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala 145 150 155 160

Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg 165 170 175

Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly 180 185 190

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Ala Leu Leu Glu Leu Glu Glu Pro Val Lys Val Ser Ser His Val His 100 105 110

Thr Val Thr Leu Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro 115 120 125

Cys Trp Val Thr Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro 130 135 140

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Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Lys Val Asn Gly 195 200 205

Thr Trp Leu Gln Ala Gly Val Val Ser Trp Gly Glu Gly Cys Ala Gln 210 215 220

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aag Lys 15	tgg Trp	ccc Pro	tgg Trp	cag Gln	gtg Val 20	agc Ser	ctg Leu	aga Arg	gtc Val	cac His 25	ggc Gly	cca Pro	tac Tyr	tgg Trp	atg Met 30	S	96
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Cys Val Gly Pro Asp Val Lys Asp Leu Ala Ala Leu Arg Val Gln Leu 50 55 60

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Ile Ile Val His Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile 85 90 95

Ala Leu Leu Glu Leu Glu Glu Pro Val Lys Val Ser Ser His Val His
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Thr Val Thr Leu Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro 115 120 125

Cys Trp Val Thr Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro 130 135 140

Pro Pro Phe Pro Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His 145 150 155 160

Ile Cys Asp Ala Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val 165 170 175

Arg Ile Val Arg Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Asp 180 185 190

Ser Cys Gln Gly Asp Ala Gly Gly Pro Leu Val Cys Lys Val Asn Gly 195 200 205

Thr Trp Leu Gln Ala Gly Val Val Ser Trp Gly Glu Gly Cys Ala Gln 210 225 220

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atg ccg tgc tgg gtc act ggc tgg ggc gat gtg gac aat gat gag cgc Met Pro Cys Trp Val Thr Gly Trp Gly Asp Val Asp Asn Asp Glu Arg 130 135 140	432
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gcc cag ccc aac cgg cct ggc atc tac acc cgt gtc acc tac tac ttg Ala Gln Pro Asn Arg Pro Gly Ile Tyr Thr Arg Val Thr Tyr Tyr Leu 225 230 235	720
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Ser Leu Arg Val His Gly Pro Tyr Trp Met His Phe Cys Gly Gly Ser

25

20

Page 23

96

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				gcc Ala 55								192
				ctg Leu								240
				cag Gln								288
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Asp Val Lys Asp Leu Ala Ala Leu Arg Val Gln Leu Arg Glu Gln His 50 60

Leu Tyr Tyr Gln Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His 65 70 75 80

Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu 85 90 95

Leu Glu Glu Pro Val Lys Val Ser Ser His Val His Thr Val Thr Leu 100 105 110

Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr 115 120 125

Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Phe Pro 130 135 140

Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala 145 150 155 160

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Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly 180 185 190

Asp Ser Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln 195 200 205

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cca cag ttc tac acc gcc cag atc gga gcg gca atc gcc ctg ctg gag Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Ala Ile Ala Leu Leu Glu 85 90 95	288
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Gly	Trp 130	Gly	Asp	Val	Asp	Asn 135	Asp	Glu	Arg	Leu	Pro 140	Pro	Pro	Phe	Pro	
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35 40 45

Asp Val Lys Asp Leu Ala Ala Leu Arg Val Gln Leu Arg Glu Gln His 50 55 60

Leu Tyr Tyr Gln Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His 65 70 75 80

Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu 85 90 95

Leu Glu Glu Pro Val Lys Val Ser Ser His Val His Thr Val Thr Leu 100 105 110

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Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg 165 170 175

Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly 180 185 190

Asp Ala Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln
195 200 205

Ala Gly Val Val Ser Trp Gly Glu Gly Cys Ala Gln Pro Asn Arg Pro Page 30

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ctc atc cac Leu Ile His 35	ccc cag tgg Pro Gln Trp	gtg ctg acc Val Leu Thr 40	gca gcg cac Ala Ala His	tgc gtg gga Cys Val Gly 45	ccg 144 Pro
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ctc tac tac Leu Tyr Tyr 65	cag gac cag Gln Asp Gln 70	ctg ctg ccg Leu Leu Pro	gtc agc agg Val Ser Arg 75	atc atc gtg Ile Ile Val	cac 240 His 80
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Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu 85 90 95

70

Leu Gl	u Glu	Pro 100	Val	Lys	Val	Ser	Ser 105	His	Val	His	Thr	Val 110	Tnr	Leu	
Pro Pr	o Ala 115	Ser	Glu	Thr	Phe	Pro 120	Pro	Gly	Met	Pro	Cys 125	Trp	Val	Thr	
Gly Tr 13		Asp	Val	Asp	Asn 135	Asp	Glu	Arg	Leu	Pro 140	Pro	Pro	Phe	Pro	
Leu Ly 145	s Gln	Val	Lys	Val 150	Pro	Ile	Met	Glu	Asn 155	His	Ile	Cys	Asp	Ala 160	
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Cys	Trp 130	Val	Thr	Gly	Trp	Gly 135	Asp	Val	Asp	Asn	Asp 140	Glu	Arg	Leu	Pro	
Pro 145	Pro	Phe	Pro	Leu	Lys 150	Gln	Val	Lys	Val	Pro 155	Ile	Met	Glu	Asn	His 160	
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Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu 90

Leu Glu Glu Pro Val Asn Val Ser Ser His Val His Thr Val Thr Leu

Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr

Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Phe Pro

Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala

Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg 170

Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly 180

Asp Ser Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln 200

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ctc atc cac ccc Leu Ile His Pro 35	cag tgg gtg Gln Trp Val	ctg acc gca Leu Thr Ala 40	gcg cac tgc Ala His Cys 45	gtg gga Val Gly	ccg 144 Pro
gac gtc aag gat Asp Val Lys Asp 50	ctg gcc gcc Leu Ala Ala 55	ctc agg gtg Leu Arg Val	caa ctg cgg Gln Leu Arg 60	gag cag Glu Gln	cac 192 His
ctc tac tac cag Leu Tyr Tyr Gln 65	gac cag ctg Asp Gln Leu 70	ctg ccg gtc Leu Pro Val	agc agg atc Ser Arg Ile 75	atc gtg Ile Val	cac 240 His 80
cca cag ttc tac Pro Gln Phe Tyr	acc gcc cag Thr Ala Gln 85	atc gga gcg Ile Gly Ala 90	gca atc gcc Ala Ile Ala	ctg ctg Leu Leu 95	gag 288 Glu
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Leu Glu Glu Pro Val Asn Val Ser Ser His Val His Thr Val Thr Leu 100 105 110

Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr 115 120 125

Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Phe Pro 130 135 140

Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala 145 150 155 160

Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg 165 170 175

Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly
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Asp Ser Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln 195 200 205

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48

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ggc Gly	tgg Trp 130	ggc Gly	gat Asp	gtg Val	gac Asp	aat Asn 135	gat Asp	gag Glu	cgc Arg	ctc Leu	cca Pro 140	ccg Pro	cca Pro	ttt Phe	cct Pro	432
ctg Leu 145	aag Lys	cag Gln	gtg Val	aag Lys	gtc Val 150	ccc Pro	ata Ile	atg Met	gaa Glu	aac Asn 155	cac His	att Ile	tgt Cys	gac Asp	gca Ala 160	480
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Asp Val Lys Asp Leu Ala Ala Leu Arg Val Gln Leu Arg Glu Gln His 50 55 60

Leu Tyr Tyr Gln Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His 65 70 75 80

Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu 85 90 95

Leu Glu Glu Pro Val Asn Val Ser Ser His Val His Thr Val Thr Leu 100 105 110

Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr 115 120 125

Gly Trp Gly Asp Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Phe Pro 130 135

Leu Lys Gln Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala 145 150 150 160

Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg 165 170 175

Asp Asp Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly 180 185 190

Asp Ala Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln Page 50

Ala Gly Val Val Ser T 210	rp Gly Glu Gly 215	Cys Ala Gln Pro Ası 220	n Arg Pro
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ctc tac tac cag gac c Leu Tyr Tyr Gln Asp G 65 7	ag ctg ctg ccg ln Leu Leu Pro	gtc agc agg atc atc Val Ser Arg Ile Ile 75	c gtg cac 240 e Val His 80
cca cag ttc tac acc g Pro Gln Phe Tyr Thr A 85			
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- Tyr Ala Ala Pro Ala Pro Val Gln Ala Leu Gln Gln Ala Gly Ile Val 20 25 30
- Gly Gly Gln Glu Ala Pro Arg Ser Lys Trp Pro Trp Gln Val Ser Leu 35 40 45
- Arg Val Arg Asp Arg Tyr Trp Met His Phe Cys Gly Gly Ser Leu Ile 50 55 60
- His Pro Gln Trp Val Leu Thr Ala Ala His Cys Leu Gly Pro Asp Val Page 55

80

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Tyr Gln Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His Pro Gln
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Phe Tyr Ile Ile Gln Thr Gly Ala Asp Ile Ala Leu Leu Glu Leu Glu
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Glu Pro Val Asn Ile Ser Ser Arg Val His Thr Val Met Leu Pro Pro 130 135 140

Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr Gly Trp 145 150 155 160

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His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Ile Arg Asp Asp 195 200 205

Met Leu Cys Ala Gly Asn Ser Gln Arg Asp Ser Cys Lys Gly Asp Ser 210 215 220

Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln Ala Gly 225 230 235 240

Val Val Ser Trp Asp Glu Gly Cys Ala Gln Pro Asn Arg Pro Gly Ile 245 250 255

Tyr Thr Arg Val Thr Tyr Tyr Leu Asp Trp Ile His His Tyr Val Pro 260 265 270

Lys Lys Pro 275